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Frequency of hepatitis B virus-DNA among hepatitis B surface-Ag negative, anti-hepatitis B core antibody-positive blood donors in Yazd, Iran

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Abstract:

BACKGROUND: The diagnosis of hepatitis B infection in most blood transfusion centers is based on hepatitis B surface (HBs) antigen detection by an enzyme immunoassay method. This study aimed to determine the frequency of hepatitis B core (HBc) antibody, HBs antibody, and hepatitis B virus (HBV) DNA among HBs antigen-negative healthy blood donors of Yazd province, Iran.

MATERIALS AND METHODS: This cross-sectional study was conducted on 1500 healthy blood donor samples negative for HBs antigen, hepatitis C virus antibody, human immunodeficiency virus antigen/antibody, and rapid plasma regain tests. All samples were screened for HBc antibody test. HBs antibody and real-time polymerase chain reaction were performed for HBc antibody-positive samples.

RESULTS: HBc antibody was positive in 74 (4.9%) samples and 11 (14.9%) of 74 positive samples for HBc antibody were negative for HBs antibody. Sixty-three (85.1%) positive samples for HBc antibody had HBs antibody titer over 10 IU/L, and 43 (58.1%) had HBs antibody titer over 100 IU/L. There was no hepatitis B DNA-positive sample in the present study.

CONCLUSIONS: The study results suggest that there is a very low risk for transmission of HBV through blood donors of Yazd, Iran.

Keywords:

Anti-hepatitis B core antibody, anti-hepatitis B surface antibody, blood donor, hepatitis B virus-DNA

Introduction

One of the most important concerns of blood transfusion centers worldwide is the transmission of hepatitis B infection through blood components. In Iran, blood transfusion centers, all of the donated blood is tested with seroprevalence hepatitis B surface (HBs)-Ag test by ELISA method. Blood units with reactive results are discarded and the confirmatory test is done if they are repeatedly reactive. Although this serologic method reduces the chance of hepatitis B transmission, studies have shown

that HBsAg-negative blood components could lead to hepatitis B infection. Hepatitis B virus (HBV) infection may lead to acute, chronic, or sometimes occult hepatitis. Occult hepatitis B is defined as the presence of HBV-DNA in blood or liver without detectable HBsAg with or without hepatitis B core (anti-HBc) antibody.

Occult HBV infection may represent (i) acute infection in the window period, (ii) HBV tail-end of chronic HBV infection, (iii) persistence of replication at low level after recovery in the presence of anti-HBs antibody, or (iv) occurrence of an escape mutant in vaccinated or

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unvaccinated individuals not detected by current HBsAg assays.^[1,2] These conditions could, therefore, lead to transfusion-transmitted hepatitis B in countries including Iran where HBV-DNA testing is not routine. The determination of the frequency of occult hepatitis in healthy blood donors is valuable for estimating the chance of HBV transmission through blood transfusion and thus change of safety strategies and screening tests.^[3]

According to previous studies, the prevalence of occult hepatitis varies in different provinces of Iran. The prevalence rate in India, Pakistan, and Turkey is 0.15%, 3%, and 0%, respectively.^[4-12] Since there are no available data about the frequency of anti-HBc antibody and HBV-DNA in blood donors of Yazd, the present study aimed to investigate the frequency of HBV-DNA among anti-HBc antibody-positive blood donors of Yazd province.

Materials and Methods

In this cross-sectional study, 1500 healthy blood donor's samples negative for HBsAg, hepatitis C virus (HCV)-Ab, HIV-Ag/Ab, and rapid plasma reagin were enrolled from 24 June to 13 May 2015. All of the blood samples were examined for anti-HBc antibody testing. Anti-HBc antibody-positive samples were tested for anti-HBs antibody and real-time polymerase chain reaction (PCR). Blood donor information was extracted from the blood transfusion software database and analysis was performed using SPSS software (IBM Corporation, New York, NY, USA).

Regular blood donor is defined as one who has donated at least two times in the past 12 months and lapsed donor is one who has donated blood >1 year ago. HBsAg, HCV-Ab, and HIV-Ag/Ab performed automatically by ELISA method ([Murex, Dartford, UK) and (Adaltis, Milan, Italia), respectively]) and anti-HBc antibody and anti-HBs antibody performed manually ([Murex, Dartford, UK] and [Siemens, Germany], respectively). DNA was extracted from anti-HBc antibody-positive serums by high pure viral nucleic acid kit (Roche, Germany). Real-time PCR was done by the TaqMan probe with a quantitative molecular diagnostic kit (Altona, Hamburg, Germany). The kit had four standard samples that have 10⁴, 10³, 10², and 10 units of virus DNA in each microliter. The four standard

samples and one negative control sample were used in each PCR reaction. Standard samples and negative control samples were used for the determination of virus quantity and measure of background fluorescent, respectively. The sensitivity of this kit was 0.5 IU/mL. Real-time PCR equipment was rotor gene 6000 of German Corbett Company. The present research project was approved by the Ethical Committee of High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.

Results

Of the total, 96.7% were men. About 61.6% regular donors, 26% lapsed donors, and 12.4% were first-time donors. Anti-HBc antibody was positive in 74 donors (4.9%), of which 71 (96%) were male (4.9% of all male participants) and 3 (4%) were female (6% of all female participants). The frequency and characteristics of donors with anti-HBc antibody are shown in Table 1.

Of the 74 blood donors positive for anti-HBc antibody, 43 donors (58.1%) had anti-HBs antibody titer over 100 IU/L, 20 donors (27%) had anti-HBs antibody titer over 10 IU/L and <100 IU/L, and 11 donors (14.9%) had anti-HBs antibody titer under 10 IU/L [Table 2]. HBV-PCR was not positive in any of the samples.

Discussion

Knowledge about the frequency of HBV infection among blood donors is of paramount importance for implementing strategies to reduce transfusion-transmissible infections and increase blood safety.

In the present study, there was no significant difference in sexuality, marital, and donation status between blood donors with or without anti-HBc antibody. Relative seroprevalence of anti-HBc antibody positivity had an inverse relation with education that was not significant after the deletion of the age effect. There was a direct relation between relative seroprevalence of anti-HBc antibody and age ($P = <0.001$) [Table 1]. This could mean that probably older individuals lived longer with HBV that could provide the chance of viral mutation or adaptation with the host immune system and stable living with it.^[13] Various studies show that Hepatitis B prevalence is on the decrease in society.^[14-16]

Table 1: Characteristics of anti-hepatitis B core antibody positive blood donors in comparison with total study subjects

| | Male (%) | Female (%) | Married (%) | Single (%) | 18-24 years (%) | 25-34 years (%) | 35-44 years (%) | 45-54 years (%) | ≥55 years (%) | Regular donor (%) | Lapsed donor (%) | First time donor (%) |
|-------------------|----------|------------|-------------|------------|-----------------|-----------------|-----------------|-----------------|---------------|-------------------|------------------|----------------------|
| Anti-HBc antibody | 4.9 | 6 | 5.3 | 2.7 | 1.9 | 2 | 5.8 | 7.9 | 11 | 4.8 | 6.2 | 3.2 |
| Total | 97 | 3 | 85 | 15 | 7 | 36 | 30 | 20 | 7 | 62 | 26 | 12 |

HBc=Hepatitis B core

There was no HBV-DNA positive sample in this study. There have been no reports of HBV transmission from blood components in recent studies in Iran.^[17,18] Different prevalence rates of occult hepatitis have been reported in different studies. It seems that virus endemicity and virus detection processes play important roles in these different results.^[4-13,19] Allain found that most of the blood donors with occult hepatitis B were over 45-year-old.^[20] In Iran, studies in different areas showed various prevalence rates of anti-HBc antibody and HBV-DNA in healthy HBsAg-negative donors [Table 3]. These differences could be due to different infection prevalence rates in each area. An Egyptian study in 2013 on 3167 HBsAg-negative blood donors showed that 14.2% had anti-HBc antibody and 17.2% of them were HBV-DNA positive. In that study, age over 30 years and being married were the most important risk factors for the prediction of anti-HBc antibody positivity and age under 30 years was a significant risk factor for HBV-DNA positivity prediction.^[13]

Viral transmission can be associated with extremely low levels of HBV-DNA in only anti-HBc antibody-positive units or blood collected during the early phase of acute infection, in which neither HBsAg nor HBV-DNA is detectable.^[21-23] In the present study, about 60% of anti-HBc antibody-positive blood donors had anti-HBs antibody titer higher than 100 IU/L and 85% had anti-HBs antibody titer higher than 10 IU/mL. There was no significant difference between different sexes, age, marital, educational, and donation status in anti-HBs antibody titer [Table 2]. Studies showed that high titer

of anti-HBs antibody had protective effect against viral infection.^[24,25] Countries such as Germany, Austria, and Japan allow transfusion of units with anti-HBs antibody titer higher than 100 IU/L.^[26] Satake *et al.*, in Japan, stated that there was no document of HBV infection in blood donors that had anti-HBc antibody and anti-HBs antibody together.^[27] Karimi *et al.* research results were also similar to the present study results; The seroprevalance rate of anti-HBc antibody was 4.9% and NAT test detects no HBV DNA.^[28]

Conclusions

The frequency of anti-HBc antibody positivity in Yazd blood donors was not high, and most of them had anti-HBs antibody titer over 100 IU/L that suggests it is not infective. There was no positive HBV-DNA sample in the study. Therefore, Yazd blood donors are low risk for transmission of hepatitis B infection.

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Conflicts of interest

There are no conflicts of interest.

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Table 2: Titer of anti- hepatitis B surface antibody in anti-hepatitis B core antibody positive blood donors

| Anti-HBs antibody titer (IU/L) | n (%) |
|--------------------------------|----------|
| Anti-HBs antibody ≤ 10 | 11 (15) |
| 10 < anti-HBs antibody < 100 | 20 (27) |
| Anti-HBs antibody ≥ 100 | 43 (58) |
| Total | 74 (100) |

HBs=Hepatitis B surface

Table 3: Prevalence of anti- hepatitis B core antibody and hepatitis B virus-DNA in anti- hepatitis B core antibody positive study samples in various cities of Iran

| City | Anti-HBc antibody (%) | HBV-DNA (%) | Reference number |
|------------------------------|-----------------------|-------------|------------------|
| Kerman | 8 | 0.02 | [4] |
| Arak | 2.1 | 0 | [5] |
| Rafsanjan | 5.2 | 0.2 | [6] |
| Esfahan | 8 | 0.01 | [7] |
| Shiraz | 6.5 | 0.01 | [8] |
| Tehran | 8 | 4 | [9] |
| Rasht | 3.8 | 1.3 | [20] |
| Yazd (hemodialysis patients) | 2.4 | 0.8 | [21] |

HBV=Hepatitis B virus, HBc=Hepatitis B core

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